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Evaluation of novel fungal and nematode isolates for control of *Conotrachelus nenuphar* (Coleoptera: Curculionidae) larvae

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Abstract

The primary objective was to identify potential nematode and fungus pathogens for control of plum curculio (*Conotrachelus nenuphar*) larvae. Initial bioassays were conducted in the laboratory. Seventeen isolates (13 wild-types and four color mutants) of the entomopathogenic fungus *Metarhizium anisopliae* var. *anisopliae* were screened. Seven of the isolates were highly virulent for plum curculio larvae ($LT_{50} = 4.0$–5.4 days at a concentration of 10^7 conidia ml^{-1}). Of these isolates, four were color mutants induced by UV-B irradiation exposure (DWR 180, 145, 142, and 62), and three of these showed significantly higher virulence than their parental wild-types (ARSEF 2575 and 23). The most virulent wild-type was the isolate ARSEF 1187. Surprisingly, higher virulence was not correlated with higher conidial adhesion to the cuticle of plum curculio larvae. A Utah-collected isolate of *Heterorhabditis bacteriophora* was as effective as a commercial strain of *Steinernema feltiae* in killing plum curculio larvae in laboratory bioassays. Larval mortality ranged from 21 to 89% at inoculum concentrations of 0.125 to 4.0×10^6 infective juveniles (IJs) per m^{-2}. A diapausing (northern) population of plum curculio was less susceptible to *S. feltiae* ($LC_{50} = 8.6 \times 10^5$ IJs m^{-2}) than a non-diapausing (southern) population ($LC_{50} = 3.6 \times 10^5$ IJs m^{-2}). A time delay in adding plum curculio larvae to sterilized soil treated with *S. feltiae* significantly reduced insect mortality after 2 days and reduced mortality below 50% after a delay of 7 days. In field tests, *S. feltiae* killed 22–39% of northern plum curculio larvae at concentrations of 0.5–2.0×10^6 IJs m^{-2}.

Keywords: Biological control; Entomopathogens; *Heterorhabditis bacteriophora*; Irradiation; *Metarhizium anisopliae*; Mutation; Plum curculio; *Steinernema feltiae*; Virulence

1. Introduction

The plum curculio, *Conotrachelus nenuphar* (Herbst), is a native pest of rosaceous fruits in eastern North America (Armstrong, 1958). Since about 1980, an isolated population has been confirmed in Box Elder County in northern Utah, USA (D.G.A., unpublished data). The primary infested sites are fruit trees in home yards and wild plum thickets. Development of efficacious biological controls is a priority for suppressing plum curculio densities in non-orchard sites, where conventional orchard practices [e.g., chemicals, crop habitat manipulations (Racette et al., 1992)] are prohibitive or impractical. During summer, fourth-instar larvae are vulnerable to entomopathogens as they exit fruit and burrow into the soil; adults are susceptible upon emergence from pupae in the soil (Armstrong, 1958). In the spring, adults may be exposed to entomopathogens in the soil as they walk to host trees from over wintering

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sites (Yonce et al., 1995) or after dropping from tree canopies to the ground upon disturbance which is a frequent behavior (Prokopy et al., 1999).

A wide variety of entomopathogens are available for control of insect pests of tree fruits, including curculionids and other coleopterans (Lacey and Shapiro-Ilan, 2003). There is ample evidence for the efficacy of entomopathogenic nematodes (Olthof and Hagley, 1993; Shanks and Agudelo-Silva, 1990; Shapiro-Ilan et al., 2002; Stuart et al., 2004) and fungi (Shapiro-Ilan et al., 2003, 2004a; Tedders et al., 1982) for suppression of curculionids. In laboratory bioassays, plum curculio larvae were susceptible to *Steinernema feltiae* (Filipjev), *S. riobrave* Cabanillas, Poinar and Raulston, and *S. carpocapsae* (Weiser) (Olthof and Hagley, 1993; Shapiro-Ilan et al., 2002) whereas *S. riobrave* and *S. carpocapsae* showed the most potential for controlling adults (Shapiro-Ilan et al., 2002). In peach and apple orchard plots, *S. riobrave* applied to soil reduced emergence of plum curculio adults by 97% (Shapiro-Ilan et al., 2004b) and *S. carpocapsae* killed 73% of larvae (Olthof and Hagley, 1993), respectively. *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin were both efficacious on plum curculio larvae (60–100% mortality) in laboratory tests (Tedders et al., 1982). There is no information on field efficacy of entomopathogenic fungi for plum curculio, but fungi have been associated with other curculionid pests of orchards. Shapiro-Ilan et al. (2003) recovered 16 isolates of *M. anisopliae* and *B. bassiana* from orchard sites in the southeastern USA and found one isolate of each fungal species caused >60% mortality of the pecan weevil [*Curculio caryae* (Horn)].

In field tests, plum curculio life stages would not be uniformly exposed to entomopathogens at the time of application. There are typically time delays following treatment applications until susceptible life stages emerge and/or move to a site where they can contact the pathogen. Therefore, it is important to assess the effect of time delay intervals on insect mortality to better predict insect control potential in the field. Olthof and Hagley (1993) found that a delay of 2 days between application of *S. carpocapsae* [at 10^5 infective juveniles (IJs) m^{-2}] and plum curculio did not reduce mortality of larvae as compared to no time delay in orchard ground cover plots. Previous research on plum curculio control with entomopathogens has been conducted exclusively with the non-diapausing, southern population of the weevil. We would expect the susceptibility of the obligate diapausing, northern population to entomopathogens to be similar to that of the southern population. Studies comparing virulence of pathogens in the two populations would be useful to test this hypothesis.

The primary objectives of the study were to compare the efficacy of a Utah strain of *Heterorhabditis bacteriophora* (Poinar) to a commercial strain of *Steinernema feltiae* and to evaluate the efficacy of 17 isolates (wild-types and UV-B mutants) of *M. anisopliae* var. *anisopliae* for control of plum curculio late fourth-instar larvae. Secondary objectives for fungus studies were to test whether virulence was related to conidial adhesion or sporulation on host larvae. Secondary objectives for nematode tests were to evaluate the effect of time-delay in exposing larvae to IJ-treated soil, if northern and southern populations of plum curculio differ in their susceptibility, and mortality of larvae in field microplots.

2. Materials and methods

2.1. Plum curculio populations

Adults and larvae of a non-diapausing population of plum curculio originally collected in Georgia, USA and maintained as a colony by Guy Hallman, USDA ARS, Weslaco, Texas, USA were obtained to initiate a laboratory population in the Department of Biology, Logan, Utah, USA. Henceforth this population is referred to as the "southern population." Plum curculio was cultured on organically grown green, thinning apples and plums (Smith, 1957). Bioassays were conducted from 6 to 18 months following introduction of field-collected insects into the colony to maintain genetic diversity. Colony size was maintained at approximately 600–800 adults and 3000–4000 larvae throughout the bioassays.

Larvae of obligate diapausing plum curculio were collected from wild plum (*Prunus americana* Marsh.) and cultivated pome [*Malus* spp. (apple and crabapple) and *Pyrus communis* L. (pear)] and stone [*Prunus avium* L. (sweet cherry), *P. cerasus* L. (sour cherry), and *P. persica* (L.) Batsch (peach)] fruits. Infested fruit was collected from Brigham City, Utah, USA during a 4-week period in June and July, 2002. Henceforth this population is referred to as the "northern population." Approximately 3600 infested fruits were placed on wire frames and approximately 1700 late fourth-instar larvae were collected into plastic trays as they dropped from fruits. Larvae were used in bioassays within 2 days after exiting fruit.

2.2. Nematode cultures and production of IJs

Steinernema feltiae was obtained from BioLogic (Willow Hill, PA, USA; originally collected by Albert Pye) and *H. bacteriophora* was collected from soil infested with plum curculio in Brigham City, Utah, USA. *H. bacteriophora* were trapped in the last instar larvae of *Galleria mellonella* (L.) placed in soil using techniques described by Kaya and Stock (1997). *H. bacteriophora* collected from Utah soil was identified by Patricia Stock, University of Arizona, Arizona, USA. Both nematode species were maintained on *G. mellonella*

larvae at room temperature (24 ± 2 °C) for use in all nematode experiments. Inoculum consisted of IJs harvested from *G. mellonella* cadavers infected 10–15 days previously using methods described by Kaya and Stock (1997).

2.3. Fungal isolates and production of conidia

Isolates of *M. anisopliae* were obtained from the USDA-ARS collection of entomopathogenic fungal cultures (ARSEF, US Plant, Soil and Nutrition Laboratory, Ithaca, NY, USA), from EcoScience (ESC, East Brunswick, NJ, USA), from the Department of Genetics and Evolution at the State University of Campinas (UNICAMP, Campinas, SP, Brazil), and from D.W. Robert's collection of *M. anisopliae* mutants (DWR, Utah State University, Logan, UT, USA). The color mutants DWR 62, 142, 145, and 180 were induced by UV-B radiation exposure (Braga et al., 2001) and have been deposited at ARSEF as accession numbers ARSEF 6844, 6989, 6991, and 6998, respectively. Table 1 presents the different isolates, their original hosts, and geographic origin.

The isolates were grown on 23 ml potato dextrose agar medium (Difco Laboratories, Sparks, MD, USA) supplemented with 1% Difco yeast extract (PDAY) in petri dishes (95 × 15 mm) in the dark at 28 ± 0.5 °C for 14 days. Conidia were harvested and suspended in 5 ml sterile 0.1% (v/v) Tween 80 solution in 15 ml tubes (modified polystyrene, Corning, Corning, NY, USA). The suspension was shaken vigorously using a vortex mixer and conidia concentrations were quantified microscopically using a hemocytometer. The dilutions were made with the 0.1% (v/v) Tween 80 solution and the concentration was adjusted to 10^7 conidia ml^{-1}. This suspension was immediately used in the bioassay testing against plum curculio larvae.

To test viability of conidia, 20 µl of a suspension (10^4 conidia ml^{-1}) were placed on petri dishes (60 × 15 mm) containing 8 ml PDAY medium. The plates were incubated in the dark at 28 °C. Percentage germination within a sample of 300 conidia per drop was determined 12 h later by counting conidia under a microscope at 400× magnification.

2.4. Entomopathogenic nematode bioassays

All laboratory bioassays (species virulence and time-delay experiments) were conducted in plastic cups (11 cm inside diameter × 11.5 cm deep) at room temperature. Sterile potting soil (Turf King, Cincinnati, OH, USA; classified as an organic matter soil, pH 6.9, and EC 5.90 dS/m) was placed into cups to a depth of 6 cm. Distilled water was added to soil to achieve 10% by weight. Desired nematode concentrations were added to 25 ml distilled water, mixed well, and poured onto the soil surface. Final soil moisture was 15% by weight. Ten plum curculio larvae were added to the soil surface of each cup. If after 5 min a larva had not burrowed into the soil, it was replaced with another.

Entomopathogenic nematode concentrations used to determine the LC_{50} and LC_{90} for *S. feltiae* and *H. bacteriophora* against plum curculio larvae were 0, 0.125, 0.25, 0.5, 1.0, 2.0, and 4.0×10^6 IJs m^{-2}. Nematode concentrations were replicated four times within a trial and trials were repeated three (northern population) or four (southern population) times on separate dates. Lab bioassays to evaluate the effect of time delay (0, 1, 2, 4, or 7 days) in exposing plum curculio larvae (southern population only) to *S. feltiae* (0 or 2.0 ± 10^6 IJs m^{-2}) on insect mortality were repeated four times on separate dates. Mortality of plum curculio larvae was determined visually after 7 days exposure.

Table 1
Geographic origin and original host of *M. anisopliae* var. *anisopliae* isolates used in bioassays against *C. nenuphar*

M. anisopliae isolates	Host/substrate	Geographic origin
ARSEF 23	*Conoderus* sp. [Coleoptera: Elateridae]	USA
ARSEF 1187	*Oxycanus* sp. [Lepidoptera: Hepialidae]	New Zealand
ARSEF 2038	*Nilaparvata lugens* (Stal) [Homoptera: Delphacidae]	Korea
ARSEF 2341	*Scotinophara coarctata* (Fabricius) [Hemiptera: Pentatomidae]	Philippines
ARSEF 2575	*Curculio caryae* (Horn) [Coleoptera: Curculionidae]	USA
ARSEF 3609	*Patanga succincta* (L.) [Orthoptera: Acrididae]	Thailand
ARSEF 4295	Soil	Australia
ARSEF 4343	Soil	Australia
ARSEF 4570	Soil	Australia
ARSEF 5626	*Tenebrio molitor* L. [Coleoptera: Tenebrionidae]	Finland
ESC 1	EcoScience (EPA registration # 129056)	USA
DWR 62	Color mutant (violet) from strain ARSEF 2575	USA
DWR 142	Color mutant (yellow) from strain ARSEF 23	USA
DWR 145	Color mutant (violet) from strain ARSEF 23	USA
DWR 180	Color mutant (white) from strain ARSEF 23	USA
UNICAMP 38	*Mahanarva posticata* (Stal) [Homoptera: Cercopidae]	Brazil
UNICAMP 58	*Deois flavopicta* (Stal) [Homoptera: Cercopidae]	Brazil

Experiments in field microplots were conducted with the northern population of plum curculio in a wild plum site in Brigham City from June 25 to July 30, 2002. *S. feltiae* was selected for evaluation because identification of the native *H. bacteriophora* population had not been confirmed. Collection of plum curculio larvae for bioassays from the field was limited to a 4-week period and we were unable to initiate *H. bacteriophora* experiments during this brief time. Microplots were made from 15 cm diam × 13.5 cm deep plastic pots with the bottoms removed and replaced with fine mesh wire cloth (1.5 mm opening). The wire cloth allowed water to flow out of the pot but prevented insect larvae from escaping. Pots were filled with native field soil (fine sandy loam; 56% sand, 35% silt, and 9% clay) and distilled water was added and mixed into soil to reach 10% moisture by weight. Microplot treatments were an untreated control, *S. feltiae* concentrations of 0.5, 1.0, and 2.0×10^6 IJs m^{-2}, and Diazinon 5G (Hi-Yield Chemical, Bonham, TX, USA) at 0.49 g active ingredient (ai) m^{-2} (mid-label rate). The granular diazinon formulation was selected for an insecticide comparison because this is the most common soil-applied insecticide in home yard sites where plum curculio occurs in northern Utah. Nematode concentrations or diazinon granules were added to 50 ml distilled water, mixed well, and poured onto the soil surface of microplots. Final soil moisture was 15–18% by weight. Ten plum curculio larvae were added to the soil surface of each pot. If after 5 min. a larva had not burrowed into the soil, it was replaced with another. Plum curculio mortality was evaluated 7 days after treatment. Aliquots of 50 ml distilled water were added to plots two or three times during the experiment when soil was dry. Tests were repeated three times on separate dates each consisting of five replications of the treatments.

2.5. Metarhizium anisopliae bioassays

Thirty plum curculio larvae from the southern population were exposed to each *M. anisopliae* isolate using an immersion method. Larvae were wrapped in sterile gauze, dipped in the conidial suspension for 15 s, and placed on sterile filter paper to absorb the excess suspension. Control larvae were dipped in Tween 80 (0.1% v/v). After treatment, larvae in groups of five were placed inside sterilized plastic cups (50 × 45 mm) with 5 g sieved (1 mm pore) sterile potting soil. The soil was moistened to 10% by weight with sterile distilled water. The cups with insects were closed with cheesecloth and maintained in glass jars with three layers of moistened filter paper. The jars were tightly sealed with Parafilm to maintain relative humidity at approximately 100% and the jars were kept at 24 °C. Mortality was assessed daily by searching through the soil, placing dead larvae in petri dishes (60 × 15 mm) with filter paper, and enclosing in humidity chambers (100% RH) at 28 °C for sporulation. Only the cadavers that produced conidia were considered to have died from mycosis. Treatments were replicated three times and mortality was recorded daily for 12 days. Trials were repeated three times on separate dates resulting in a total of 90 larvae exposed to each isolate. The isolate ARSEF 2575 was used as a standard comparison because it was originally isolated from another curculionid, *C. caryae*.

To determine conidial adhesion on insect bodies, three additional plum curculio larvae were exposed to conidial suspensions of each fungal isolate as before, immersed in 300 µl of Tween 80 (0.1% v/v) in Eppendorf tubes, vortexed for 15 s, and the number of conidia estimated using a hemocytometer. Trials were replicated three times resulting in a total of 9 larvae tested for each isolate.

2.6. Data analyses

All experimental designs were a randomized complete block with trial date as the block. Mortality data were corrected for control mortality (Abbott, 1925). No mycosis or nematode infection was observed in control larvae. Probit analysis (SAS Institute, 2002) was used to calculate lethal concentrations (LC_{50} and LC_{90}) for nematodes and median lethal time (LT_{50}) for fungal isolates. Significant differences were based on failure of 83% confidence intervals (CIs) to overlap. Payton et al. (2003) showed that 83% fiducial limits most closely approximate a 0.05 test for LC_{50} and LC_{90} comparisons among insect populations. One-way analyses of variance (PROC GLM; SAS Institute, 2002) were used to test for differences among treatments in nematode time-delay and field experiments and for fungus conidial adhesion tests. Means were separated with Tukey's procedure ($\alpha = 0.05$). Pearson correlation coefficient was calculated to test for correlation between conidial adhesion and virulence of *M. anisopliae* isolates at 4 and 5 days after infection (PROC CORR; SAS Institute, 2002). The difference between mycosis-confirmed and corrected mortality for fungal isolates was assessed with a two-way analysis of variance (PROC GLM; SAS Institute, 2002). The means for each isolate were compared using a contrast; this set of 17 contrasts was not adjusted for experiment-wise Type I error. Percent mortality data were arcsine square-root transformed and conidial adhesion data were square-root transformed prior to analysis to meet assumptions of normality and homogeneity of variance. Non-transformed means and standard errors (SE) are presented.

3. Results

3.1. Efficacy of entomopathogenic nematodes

Mean corrected mortality of plum curculio ranged from 20.9 to 88.7% for the two species of nematodes and

two weevil populations across inoculum concentrations tested (0.125 to 4.0 × 10^6 IJs m^{-2}) (Fig. 1). The southern population responded similarly to *S. feltiae* and *H. bacteriophora* and LC_{50} and LC_{90} values were similar between nematode species (Table 2). The northern population of was less susceptible to *S. feltiae* than the southern population was to either nematode species for inoculum concentrations from 0.25 to 2.0 × 10^6 IJs m^{-2} (Fig. 1). In contrast, there was no difference in morality between populations at the highest nematode concentration tested, 4.0 × 10^6 IJs m^{-2}. LC_{50} values were greater for the northern than the southern population, but LC_{90} values did not differ among insect populations or nematode species (Table 2).

A time delay in adding plum curculio larvae following soil inoculation with *S. feltiae* significantly reduced insect mortality after 2 days and further reduced mortality by 7 days (Table 3) ($F = 16.26$; $df = 4, 99$; $P < 0.01$). Mean corrected mortality remained above 50% for up to 4 days delay.

Mortality of field-collected plum curculio larvae from the northern population differed among nematode and insecticide treatments in field microplot experiments (Fig. 2) ($F = 21.08$; $df = 3, 59$; $P < 0.01$). Mean corrected mortality was greatest for the Diazinon treatment

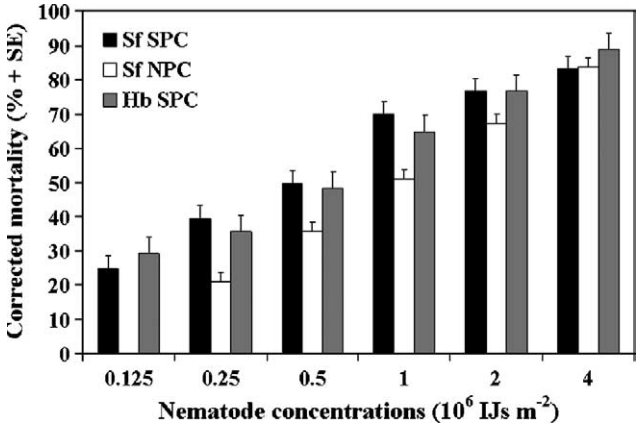

Fig. 1. Effect of inoculum concentration for two entomopathogenic nematode species (Sf, *Steinernema feltiae*; Hb, *Heterorhabditis bacteriophora*) on mortality of *C. nenuphar* larvae from two populations (SPC, southern population; NPC, northern population) in laboratory bioassays. Mean control mortality was 5.0 ± 1.8%, 3.3 ± 1.4%, and 10.6 ± 2.3% for Sf SPC, Sf NPC, and Hb SPC, respectively.

Table 3
Mean corrected mortality of *C. nenuphar* larvae (Southern population) as influenced by time delays in introducing the insect to sterilized soil with *S. feltiae* (2.0 × 10^6 IJs m^{-2})

Delay (days)	Mean corrected mortality (%±SE)
0	75.2 ± 2.4 a
1	74.2 ± 3.0 a
2	61.2 ± 3.5 b
4	51.4 ± 4.5 b
7	38.1 ± 4.9 c

Corrected mortality means followed by the same letters are not significantly different (Tukey's test, $\alpha = 0.05$). Mean control mortality = 3.5 ± 2.0%.

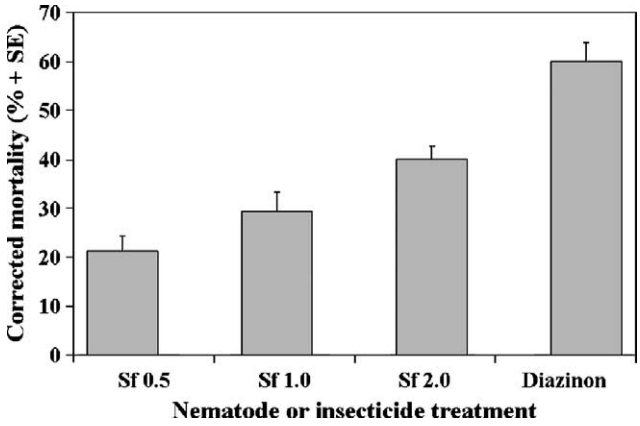

Fig. 2. Influence of application of an insecticide (Diazinon 5G at 0.49 g ai m^{-2}) or *Steinernema feltiae* (Sf) concentrations (0.5, 1.0, and 2.0 × 10^6 IJs m^{-2}) on mortality of northern *C. nenuphar* larvae in field microplot trials. Means were separated with a Tukey's test ($\alpha = 0.05$). Mean control mortality was 17.3 ± 2.9%.

(60.7 ± 3.8%), second highest for *S. feltiae* applied at 2.0 × 10^6 IJs m^{-2} (40.0 ± 2.8%) and lowest for *S. feltiae* applied at 0.5 and 1.0 × 10^6 IJs m^{-2} (29.3 ± 4.1 and 21.3 ± 2.9%, respectively). Mortality of larvae was reduced by 40–42% in field microplot experiments as compared to laboratory bioassays for the same *S. feltiae* concentrations.

3.2. Efficacy of fungal isolates

Mean viability of conidia of all *M. anisopliae* isolates ranged between 93.0 and 99.5%. Seven isolates were the most virulent for plum curculio larvae: DWR 180, DWR

Table 2
Influence of two entomopathogenic nematode species, *S. feltiae* and *H. bacteriophora*, on the concentration needed to kill 50% and 90% (LC_{50} and LC_{90}, respectively) of *C. nenuphar* larvae from two populations

Nematode species	*C. nenuphar* population	LC_{50} (×10^5 IJs m^{-2}) (83% CI)	LC_{90} (× 10^6 IJs m^{-2}) (83% CI)
S. feltiae	Southern	3.6 (2.7–4.5) b	7.2 (4.8–12.7) a
	Northern	8.6 (7.5–9.7) a	7.0 (5.3–9.9) a
H. bacteriophora	Southern	3.3 (2.5–4.2) b	5.2 (3.5–9.6) a

Values in columns followed by different letters are significantly different based on failure of 83% CIs to overlap. Use of 83% fiducial limits was selected to approximate $\alpha = 0.05$. Mean control mortality = 5.0 ± 1.8%, 3.3 ± 1.4%, and 10.6 ± 2.3% for *S. feltiae*-Southern, *S. feltiae*-Northern, and *H. bacteriophora*-Southern, respectively.

Table 4
Effect of *M. anisopliae* var. *anisopliae* isolates on conidial adhesion, median lethal time to kill 50% of the population (LT_{50}), and relative potency to *C. nenuphar* larvae (exposed to 10^7 conidia ml^{-1})

M. anisopliae isolates	# conidia/insect[a] (±SE)				LT_{50}	83% CI[b]		Relative potency[c]
DWR 180	10.8 (4.4)	A	B	C	4.0	3.4	4.8	1.30
DWR 145	7.7 (3.8)		B	C	4.3	3.8	4.8	1.21
DWR 142	27.1 (10.6)	A			4.4	3.9	5.0	1.18
ARSEF 1187	6.3 (2.7)		B	C	4.8	4.3	5.5	1.08
DWR 62	12.5 (4.2)	A	B	C	4.9	4.4	5.4	1.06
ARSEF 2575	10.8 (2.3)	A	B	C	5.2	5.1	5.4	1.00
ARSEF 23	16.1 (5.4)	A	B		5.4	5.1	5.6	0.96
ARSEF 4570	2.5 (0.5)			C	6.1	5.7	6.5	0.85
ESC 1	7.0 (2.6)		B	C	6.3	5.7	6.9	0.83
ARSEF 5626	13.0 (4.1)		B	C	7.1	6.8	7.5	0.73
ARSEF 2341	9.4 (3.9)	A	B	C	7.3	5.3	13.8	0.71
ARSEF 4295	7.4 (2.9)		B	C	7.9	7.4	8.8	0.66
UNICAMP 38	5.9 (3.4)			C	7.9	7.6	8.3	0.66
UNICAMP 58	7.1 (3.1)		B	C	10.3	8.4	16.5	0.50
ARSEF 2038	14.0 (0.0)		B	C	13.0	11.2	22.0	0.40
ARSEF 4343[d]	6.3 (3.4)		B	C	22.8			0.23
ARSEF 3609[e]	6.2 (1.4)			C				

Mean control mortality = 14.1 ± 1.5% on 12 days after inoculation.
[a] Number of conidia per insect (× 10^4). Mean conidial counts followed by the same letters are not significantly different (Tukey's test, $\alpha = 0.05$).
[b] LT_{50} values for isolates differ when 83% confidence intervals (CI) do not overlap. Use of 83% fiducial limits was selected to approximate $\alpha = 0.05$.
[c] Relative potency = LT_{50} of standard isolate (ARSEF 2575)/LT_{50} of test isolate.
[d] 83% CI not calculable because of poor fit.
[e] Mortality caused by isolate < 50%.

145, DWR 142, ARSEF 1187, DWR 62, ARSEF 2575, and ARSEF 23 (LT_{50} = 4.0–5.4 days) (Table 4). The 83% CI of these seven isolates did not overlap with isolates of lesser virulence, except ARSEF 2341 which had a large CI. One isolate, ARSEF 3609, was non-virulent for plum curculio.

Adhesion of *M. anisopliae* conidia to plum curculio larvae varied among isolates. Conidial adhesion was significantly greater for isolate DWR 142 than for 11 other isolates ($P \leqslant 0.05$; Table 4). Adhesion in five isolates was not different from DWR 142. Conidial adhesion was least for ARSEF 3609, ARSEF 4570, and UNICAMP 38. Although some isolates with higher numbers of conidia adhesion per insect also had lower LT_{50} values, there was not a significant correlation between conidial adhesion and mortality of plum curculio larvae measured at 4 and 5 days after infection (Pearson Correlation Coefficients = 0.35 and 0.40, respectively).

Mycosis-confirmed mortality was similar to Abbott-corrected mortality for most isolates, except for DWR 142, DWR 145, and ESC-1 (contrasts; $P < 0.001$, $P < 0.001$, and $P = 0.008$, respectively) (Fig. 3). For these three isolates, the mycosis that developed in plum curculio larvae was significantly less than corrected mortality, indicating that reproduction of the fungus was low.

4. Discussion

Plum curculio larvae were effectively killed by four novel isolates of *M. anisopliae* var. *anisopliae* and the Utah-collected strain of *H. bacteriophora* was as efficacious as the commercial strain of *S. feltiae* in laboratory soil bioassays under ideal conditions (10–15% soil moisture and 24 °C). The nematode *S. feltiae* was less efficacious in field microplot studies. Results presented on the effect of time-delay exposure to nematodes, relative susceptibility of southern vs. northern plum curculio populations, and field control are preliminary and require further testing with more species of entomopathogens to form more firm conclusions.

The most virulent isolates of *M. anisopliae* var. *anisopliae* were the four color mutants induced by UV-B irradiation, DWR 180, 145, 142, and 62, and three wild-type isolates, ARSEF 1187, 2775, and 23. The LT_{50} of these seven isolates ranged from 4.0 to 5.4 days. The color mutants were obtained by UV-B irradiation of two of the most virulent wild-type isolates, ARSEF 23 and 2575 (Braga et al., 2001). It is interesting that the native host of ARSEF 1187 is from Lepidoptera, yet this isolate was more virulent to plum curculio than some isolates collected from coleopteran hosts. In addition to virulence, other important selection criteria for entomopathogens include ultraviolet and heat tolerance (Inglis et al., 1997; Braga et al., 2001; Rangel et al., 2005), and selection for rhizosphere competence, which is particularly important for insect hosts that spend part of their life cycle in the soil (Roberts and St. Leger, 2004).

Hydrophobicity of conidia is another parameter that has been suggested for use in screening fungal isolates. A high propensity for attaching to the cuticle of insects may increase infectivity (Altre et al., 1999; Jeffs and Khachatourians, 1997). We did not find a correlation

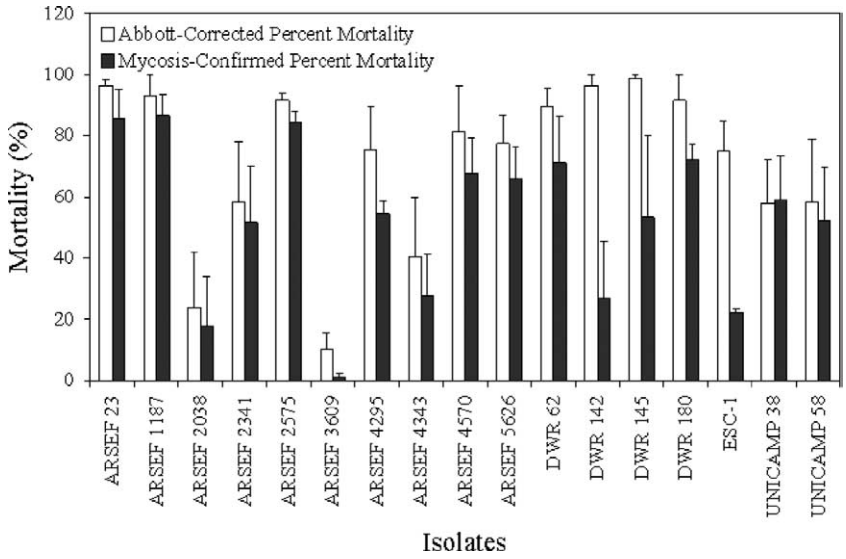

Fig. 3. Comparison of Abbott-corrected and mycosis-confirmed (sporulation observed) mortality of *C. nenuphar* larvae (southern population) infected with different isolates of *M. anisopliae* 8 days after treatment. Mean control mortality was 8.8 ± 1.6%.

between virulence and conidial adherence to plum curculio larvae across isolates; however, five of the seven most virulent isolates had the greatest mean conidial adhesion counts in the study.

Interestingly, three of the fungal mutants (DWR 180, 145, and 142) were more virulent than their parental wild-types (ARSEF 2575 and 23) based on non-overlap in 83% CIs for LT_{50}s. Relative potencies for mutants ranged from 1.06 to 1.30 as compared to 0.96 and 1.00 for their wild-type parents. Other authors have also observed higher virulence in mutants than wild-types (Al-Aidroos and Roberts, 1978; Al-Aidroos and Seifert, 1980); however, Oliveira et al. (1994) found that color mutants were less virulent to boll weevil (*Anthonomus grandis* Boheman) than their wild-types. High virulence was not related to high sporulation of strains on the insect host. Two of the color mutants had reduced mycosis as compared to non-genetically altered strains. Since the mutagenic sources utilized in this study invoke several types of mutations, we may not be able explain the relationship to virulence without a genetic study.

The native strain of *H. bacteriophora* was as efficacious for control of plum curculio as the commercial strain of *S. feltiae*. Within a practical field application rate range, 0.25 to 1.0×10^6 IJs m^{-2}, mortality of the non-diapausing, southern population of plum curculio was 35–70%. LC_{50} values were 3.3 and 3.6×10^5 IJ m^{-2} for *H. bacteriophora* and *S. feltiae*, respectively. In comparison to other studies using soil bioassays and targeting plum curculio larvae, mortality in our study was lower than for *S. feltiae* [>90% mortality at 4×10^5 IJs m^{-2} (Shapiro-Ilan et al., 2002)] and *S. carpocapsae* [(91% mortality at 10^5 IJs m^{-2} (Olthof and Hagley, 1993)]. We used a soil depth of 6 cm in bioassay containers to simulate field conditions and allow larvae to migrate downward. Lower mortality in our study may be partly explained by insect escape from nematode infection. We found the majority of successful pupae within 1 cm of the bottom of bioassay cups suggesting that larvae preferred to burrow at least 5 cm deep before pupating. In the field, pupation occurs at a depth of 1–8 cm (Quaintance and Jenne, 1912). Shapiro-Ilan et al. (2002) used a shallow soil bioassay (3.5 cm deep) that would have limited movement of larvae and perhaps increased the likelihood of infection by nematode IJs.

We observed a lower susceptibility of the obligate diapausing, northern population of plum curculio to *S. feltiae* infection ($LC_{50} = 8.6 \times 10^5$ IJs m^{-2}) than for the southern population in laboratory bioassays. However, LC_{90} values did not differ among populations or nematode species ($5.2–7.2 \times 10^6$ IJs m^{-2}), where variance in mortality was greater at higher nematode concentrations. This is the first study to evaluate susceptibility of the northern population of plum curculio to nematodes. We can speculate on several reasons for the lower susceptibility of the northern population. Exposure to a wider range of environmental conditions and diet could increase the genetic vigor of the field-collected northern population as compared to the southern population that had been reared on apple and plum for 6–18 months following introduction of field-collected insects. In addition, perhaps some behavioral differences exist between the populations, such as preferred pupation depth. We did not collect data on pupation depth, but we observed that the majority of successful pupation in both populations occurred at 5–6 cm deep in the laboratory bioassay containers.

Performance of *S. feltiae* in killing northern plum curculio larvae under field conditions was reduced by 40–42% as compared to laboratory trials. Diazinon 5G

at 0.49 g ai m^{-2} was more efficacious than any concentration of *S. feltiae*. At reasonable field application concentrations, 0.5 to 1.0×10^6 IJs m^{-2}, *S. feltiae* provided only 21–29% control of plum curculio larvae. This level of control would not be acceptable as a single control approach, but may have a fit for suppression of plum curculio in home yard and wild plum sites in combination with insecticides or other controls. In contrast to other orchard trials with plum curculio, Shapiro-Ilan et al. (2004b) and Olthof and Hagley (1993) found 97 and 73% mortality of field populations using *S. riobrave* and *S. carpocapsae*, respectively. Low soil moisture and high temperatures may have been factors in reducing nematode efficacy in our field experiments. The soil was initially dry (<5%) in our field study site and without vegetation cover. Water was added to field microplots two to three times during the 7-day trial periods to maintain moisture $\geqslant$10%. Microplots were adjacent to thickets of wild plums, but not substantially shaded by the tree canopies. Field experiments were conducted from late June through July when maximum ambient temperatures ranged from 27 to 39 °C. Further testing of the northern population with Utah *H. bacteriophora* and other nematode species that may be better adapted to Utah field conditions is needed.

In the field, plum curculio larvae may emerge from fruit and enter the soil over a 4–6-week period (Racette et al., 1992). We found that time delays between application of *S. feltiae* and plum curculio larvae lowered insect mortality after 2 days (from 75% at 0 days down to 61%), and lowered mortality further after 7 days (down to 38% mortality). In orchard sod plots, Olthof and Hagley (1993) found no reduction in infection of plum curculio larvae with *S. carpocapsae* after 2 days whether nematodes or insects were added first (73 and 76%, respectively). Our results from laboratory bioassays suggest that to maintain insect mortality above 50%, nematodes would need to be reapplied every 4 days. However, this does not consider the ability of entomopathogens to recycle in their hosts, and so their longevity in the field could be extended. Shapiro-Ilan et al. (2002) found substantial reproduction of entomopathogenic nematodes in plum curculio larval cadavers (2000–10,000 IJs produced per insect). In addition, Shapiro-Ilan et al. (2004b) showed that in peach orchards in Georgia and Florida, persistence of *S. riobrave* in soil for 3–4 weeks resulted in 77.5–100% reduction in emergence of plum curculio adults. In well watered field sites such as home yards, reapplication of nematodes may be less frequently needed because of high levels of nematode recycling. In another study, *S. feltiae* and *H. bacteriophora* were collected from soil in home yards in Brigham City, UT up to 4 weeks after inoculation (D.G.A., unpublished data).

Another approach that may enhance dispersal and longevity of entomopathogens in the environment is autodissemination, or dispersal of microbial agents in insect hosts to infect new individuals (Klein and Lacey, 1999; Vega et al., 2000). Autodissemination is more practical for adult life stages of insects, and bears further study for application to plum curculio control. Pathogen recycling and autodissemination could be a disadvantage to the use of entomopathogens from the industry standpoint. For commercial biological control agents, self-perpetuation in the environment may decrease income for the manufacturer as compared to potential profits from shorter residual toxicants. We observed that three *M. anisopliae* isolates sporulated sparingly on cadavers, especially the isolate ESC-1 registered for termite control by EcoScience Corporation. Wright et al. (2002) also found that the ESC-1 isolate sporulated in only 49% of the dead termites as compared to a non-registered isolate that sporulated on 74% of the dead termites.

In conclusion, selected isolates of both the fungal agent, *M. anisopliae* var. *anisopliae*, and the nematodes, *H. bacteriophora* and *S. feltiae*, show promise for biological control of the plum curculio. More research is needed to further test these agents under field conditions and against the northern population of plum curculio.

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